Video Article

Dual Electrophysiological Recordings of Synaptically-evoked Astroglial and Neuronal Responses in Acute Hippocampal Slices

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Abstract

Astrocytes form together with neurons tripartite synapses, where they integrate and modulate neuronal activity. Indeed, astrocytes sense neuronal inputs through activation of their ion channels and neurotransmitter receptors, and process information in part through activity-dependent release of gliotransmitters. Furthermore, astrocytes constitute the main uptake system for glutamate, contribute to potassium spatial buffering, as well as to GABA clearance. These cells therefore constantly monitor synaptic activity, and are thereby sensitive indicators for alterations in synaptically-released glutamate, GABA and extracellular potassium levels. Additionally, alterations in astroglial uptake activity or buffering capacity can have severe effects on neuronal functions, and might be overlooked when characterizing physiopathological situations or knockout mice. Dual recording of neuronal and astroglial activities is therefore an important method to study alterations in synaptic strength associated to concomitant changes in astroglial uptake and buffering capacities. Here we describe how to prepare hippocampal slices, how to identify *stratum radiatum* astrocytes, and how to record simultaneously neuronal and astroglial electrophysiological responses. Furthermore, we describe how to isolate pharmacologically the synaptically-evoked astroglial currents.

Video Link

The video component of this article can be found at http://www.jove.com/video/4418/

Protocol

1. Preparation of Artificial Cerebrospinal Fluid and Intracellular Solution

1. Before starting the experiment, one needs to prepare the internal solution for the patch clamp recordings, as well as the artificial cerebrospinal fluid (ACSF) for the hippocampus preparation. You will furthermore need a dissection kit consisting of surgical scissor and fine iris scissor, two spatulas and forceps (Fine science tools); a glass gassing device (micro-filter candle, ROBU Germany) and tissue grid (mosquito net or nylon tight), as well as superglue (Uhu Dent). The configuration of the electrophysiology slice patch setup was described by Finkel & Bookman, 2001[1].
2. For the internal solution, dissolve (in mM): 105 K-gluconate, 30 KCl, 10 HEPES and 0.3 EGTA in deionized water (in 70-80% of the final volume). Cool the solution to 4 °C and add (in mM): 4 ATP-Mg, 0.3 GTP-Tris and 10 phosphocreatine. Adjust the pH to 7.4 with KOH and fill up with deionized water to the final volume. (Osmolaritiy: ~ 280 mOsm). Filter this solution (pore size 0.2 µm). Aliquoted solution is stable for 3-4 weeks at - 20 °C. For one experimental day, ~ 1 ml of internal solution is needed.
3. Unless otherwise stated, the ACSF used for hippocampus preparation and recordings of cells in the CA1 region, contains (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$ and 11 glucose. Dissolve these salts in deionized water (Osmolarity ~ 320 mOsm) and oxygenate this solution for at least 10 min (pH ~ 7.3 - 7.4) with carbogen (95 % O_2 and 5 % CO_2). Prepare at least 1 liter of solution per experiment. Particular care should be taken for preparation of hippocampal tissue that will be used to perform experiments in the CA3 region of the hippocampus. Indeed, this region is prone to epileptiform activity and subsequent neuronal death. Thus synaptic activity should be strongly reduced during slice preparation, and this is achieved by performing the hippocampus dissection in ice-cold sucrose solution containing (in mM): 87 NaCl, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 1 NaH_2PO_4, 25 $NaHCO_3$, 10 Glucose and 75 Sucrose. In this solution, the combination of low sodium, low calcium and high magnesium concentrations massively reduce presynaptic firing and release probability, as well as postsynaptic NMDA receptor activity, thus minimizing spontaneous activity and cell death. Once prepared, hippocampal slices used for recordings of cells from the CA3 region are perfused with modified ACSF, containing 4 mM $CaCl_2$ and 4 $MgSO_4$, to minimize polysynaptic activity.

2. Acute Hippocampal Slice Preparation

1. Cool down ~ 300 ml of ACSF for the slicing chamber, as well as for the preparation at 4 °C, while constantly oxygenating with carbogen. Prepare a small beaker with ACSF at room temperature (RT) for slice storage, which is also oxygenated with carbogen (Scheme **Figure 1**).
2. Anesthetize the mouse under a hood with a small paper towel soaked with 1 ml isoflurane that is added into the cage.
3. After the mouse is deeply anesthetized, cut the head off and add it directly into a small dish with ice-cold oxygenated ACSF. Remove the scalp with a small scissor and transfer the head onto tissue for the subsequent steps. Start to dissect the hippocampus, as illustrated and described in **Figure 1**.
4. Cut 300-400 µm thick transverse slices at low speed (3 µm/sec) and vibration frequency of 70 Hz in ice cold oxygenated ACSF, and transfer them into a storage chamber. Let the slices rest at RT for at least 1 hr prior recording. Slices for CA3 experiments are stored 25 min at 34 °C and at least 30 min at RT, to recover from the slicing process.

3. Dual Recording of Evoked Astroglial Currents and Neuronal Field Potentials

We here describe how to record synaptically-evoked astroglial and neuronal responses, *i.e.* responses induced by synapse activation through afference stimulation using an extracellular electrode.

1. Constantly perfuse the recording chamber with oxygenated ACSF (1.5-2 ml/min, RT), containing 100 µM picrotoxin ($GABA_A$ antagonist) to isolate excitatory responses. Transfer a slice onto a poly-L-lysine (1.5 to 3 mg/ml) coated coverslip, soak the liquid to achieve a good slice adhesion and add a drop of ASCF on top of the slice. Place the coverslip into the recording chamber. Blockade of inhibitory transmission by picrotoxin can result in epileptiform activity, *i.e.* spontaneous, synchronous firing of neuronal populations, which will distort the measurement of evoked events. Thus, to prevent epileptiform activity, make a flat cut (only the surface) between the CA1 and CA3 regions to prevent the propagation via the Schaffer collaterals (as indicated in **Figure 2a**).
2. *Stratum radiatum* astrocytes can be identified by their small soma size (~ 10 µm) and stellate process assembly. Choose a cell at least 20-30 µm below the slice surface. Mount a glass stimulation electrode (tip resistance ~ 1 MΩ) on the silver wire that is connected to the stimulus isolation box and grounded to the bath (simply by wrapping the second silver wire around the glass pipette). Place the stimulation electrode into the Schaffer collateral region, as indicated in **Figure 2A** at a distance of 200-300 µm away from the chosen astrocyte. Mount the field recording electrode (~2-5 MΩ) onto a chlorinated silver wire connected to the headstage of the amplifier. Both electrodes are filled before with ACSF. Choose in multiclamp the mode I=0, which disables external command input and place the electrode ~ 50 µm away from the astrocyte into the *stratum radiatum* region (**Figure 2A**). Record the responses with Gain 2 to 10 and filter with a 2 kHz Bessel filter. Electric current injection into the brain slice triggers action potentials in the surrounding Schaffer collateral axons and subsequent transmitter release at the presynaptic terminals that project to postsynaptic CA1 pyramidal neurons. The released transmitters will trigger a positive charge flow into the cells through postsynaptic ionotropic receptors, which is measurable extracellularly as a small negative potential. This field excitatory postsynaptic potential (fEPSP) integrates the activity of a group of simultaneously active neurons, while inhibitory transmission is blocked pharmacologically. Apply some test pulses (0.1 msec duration) to evoke a fEPSP; some repositioning of the stimulation electrode might help to increase the fEPSP. A typical CA1 *stratum radiatum* fEPSP is illustrated in **Figure 2B**. Further details on positioning and response waveforms can be found in Yuan *et al.* 2003[2]. The fEPSP amplitude in a healthy hippocampal slice should usually be more than twice as big as the amplitude of the fiber volley. For accurate quantification of fEPSP amplitude or slope, the evoked reponse should be monosynaptic, as polysynaptic activity (detectable as a multi-peak response) indicates synaptic activity independent of the electrical stimulation, which could be a sign for hyperexcitability. For experiments performed in the CA3 region of the hippocampus, stimulation and recording pipettes are positioned as illustrated in **Figure 3C**. To clearly identify mossy fiber inputs, which are strongly facilitating, paired-pulse stimulation (50 msec interpulse interval) and 1 Hz stimulation for a few seconds are applied to massively enhance the initially low amplitude evoked fEPSP responses.At the end of the experiment, DCGIV, a mGluR2/3 receptor antagonist, can be washed in to further verify that indeed mossy fiber inputs were stimulated. Application of this antagonist should reduce the fEPSP by ~ 90% due to the high expression of mGluR2/3 receptors, inhibiting presynaptic release from mossy fiber boutons.
3. Fill a patch pipette (~ 2-5 MΩ) with filtered internal solution and mount it onto a chlorinated silver wire connected to the second headstage, apply positive pressure with a syringe, which is connected via tubing to your pipette holder. Constantly apply a 20 msec test pulse of 10 mV and move the pipette into the tissue until you reach the cell surface and a deflection in the membrane becomes visible. Zero the pipette offset, remove the positive pressure, and clamp the membrane to - 80 mV. Wait until a gigaseal (at least 1 GΩ) is reached (it should not take longer than several seconds). Gentle application of some negative pressure might help to reach a gigaseal. Break into the cell is achieved by a short application of negative pressure or using the zap function in multiclamp. Start the simultaneous recording of the Schaffer collateral evoked fEPSP and the astroglial response in voltage clamp (Vhold - 80 mV; frequency 0.1 Hz, bessel filter 2 kHz, gain 10). The astroglial current response is biphasic: first you will see a fast transient outward current, reflecting fEPSPs generated by adjacent pyramidal cells. This is followed by a slowly rising and decaying inward current (persisting several seconds (> 10 sec) after termination of neuronal responses). This current is mainly due to potassium entry into astrocytes, following release by surrounding depolarized postsynaptic terminals. A simultaneously activated fast transient glutamate transporter current (GLT), triggered by presynaptic glutamate release is masked by the potassium current. The holding potential of the astrocyte, as well as the access resistance of the patch should be monitored throughout the experiment and should not vary more than ~ 20%, to avoid inaccurate monitoring of astroglial reponses due to changes in the recording conditions. Only astrocytes with an initial holding potential > -70 mV should be investigated to study healthy cells.
4. Switch from voltage to current-clamp in order to record the evoked astrocytic membrane depolarization. Isolate the glial glutamate transporter (GLT) current by perfusion of the ionotropic glutamate receptor blocker kynurenic acid (5 mM), until the fEPSP is fully blocked and the GLT amplitude has reached a plateau. To clearly identify the GLT current, apply the specific antagonist DL-threo-β-Benzyloxyaspartatic acid (DL-TBOA, 200 µM). Increase the stimulation strength by 2-fold to 5-fold to record neuronal and astroglial responses at different synaptic strength, and apply two closely spaced stimuli (50 msec interval) to investigate responses to paired pulse stimulation. Stability of series resistance and membrane potential of the glial cell should be monitored throughout the recording.
5. To visualize the extent of the gap-junction mediated astroglial networks, dye-coupling experiments should be performed in current clamp mode, without any current injection, to enable passive diffusion of low molecular dyes (< 1.5 kDa), such as sulforhodamine-B, through gap

junction channels. To minimize dye spillover into the surrounding tissue, positive pressure should be applied through the patch pipette just when entering the tissue and the patch should be reached as soon as possible.

Representative Results

A representative simultaneous recording of synaptically-evoked astroglial and neuronal responses (fEPSPs) in the CA1 area of the hippocampus is shown in **Figure 2 A-B**. The evoked astroglial current is biphasic, i.e. it consists of a transient outward current and a slowly decaying inward current (> 10 sec) (**Figure 2B**). The outward current reflects the evoked fEPSP, and is blocked after inhibition of ionotropic glutamate receptors by kynurenic acid (dark grey trace, **Figure 2B**)[3]. The majority of the slow inward current reflects potassium entry into the astrocyte following postsynaptic depolarization, since it is also abolished by kynurenic acid, which inhibits postsynaptic ionotropic glutamate receptor activity (**Figure 2B** and **Figure 2C**$_1$)[3-6], known to represent the main source (80 %) of potassium release[7]. The remaining rapidly rising and decaying inward current is inhibited by the GLT antagonist DL-TBOA (light grey trace, **Figure 2B** and **Figure 2C**$_2$). Post-hoc subtraction of the remaining slow current in TBOA (light grey trace) from the current in kynurenic acid (dark grey trace) allows the isolation of the pure astroglial glutamate transporter current (black trace), as illustrated in **Figure 2C**$_2$. The persistent slowly decaying current in kynurenic acid and TBOA (light grey trace, **Figure 2B** and **Figure 2C**$_2$) can be blocked by TTX (data not shown), and reflects most likely the accumulation of extracellular K^+ released during presynaptic afferent firing[3]. Moderate single stimulation of Schaffer collaterals induces a relatively large synaptically-evoked astroglial current compared to the small evoked depolarization recorded in the same cell (**Figure 2D**). This is due to the low membrane resistance of astrocytes. Recording of the synaptically-evoked astroglial membrane potential dynamics, as illustrated in **Figure 2D**, is a direct measure of local extracellular potassium levels[8]. Normalization of the evoked astroglial responses to the underlying neuronal activity allows the direct comparison of different experiments, as recently shown[6]. Astroglial currents can furthermore monitor very reliably alterations in excitatory transmission, as the total synaptically-evoked astroglial current follows linearly the increase in the fEPSP (**Figure 2E**). Astroglial currents also reflect short-term synaptic plasticity, since they show, as neurons, paired-pulse facilitation (**Figure 2F**). Paired whole-cell recording of a CA1 pyramidal cell and an astrocyte reveal very different electrophysiological behavior in both cell types, since the neuron display action potentials in response to a depolarizing pulse, while the neighboring astrocyte is silent (**Figure 3A-B**). However, moderate stimulation of the Schaffer collaterals can evoke simultaneously a fast excitatory posynaptic potential in the CA1 pyramidal cell and a fast outward and slow inward currents in the adjacent astrocyte (**Figure 3B**$_2$). Dual recordings of synaptically-evoked neuronal and astroglial responses can also be recorded in the CA3 area of the hippocampus, as shown in **Figure 3C**. Indeed, single stimulation of CA3 mossy fibers evokes in basal conditions very small neuronal responses, recorded as local fEPSPs, associated to small fast outward and slow inward currents in astrocytes (**Figure 3D**$_1$). In contrast, 1 Hz stimulation of CA3 mossy fibers for a few seconds strongly potentiates the fEPSP, while it only moderately increases the astroglial response (**Figure 3D**$_2$).

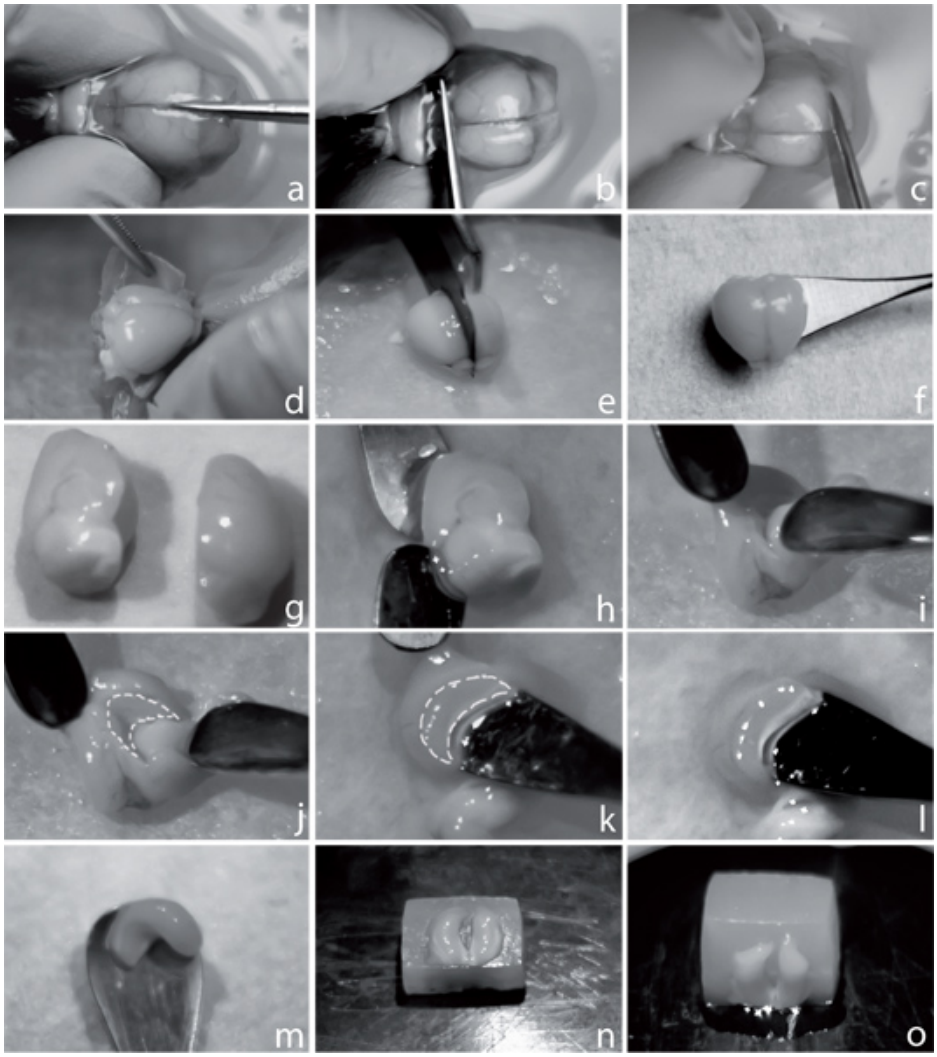

Figure 1. Hippocampus isolation to prepare transversal slices. To dissect the brain, cut the scull along the midline (a). Make a coronal cut at the level of the olfactory bulb (b) and subsequently at the level of the cerebellum (c). Carefully remove the scull with the help of a forceps (d), separate the two hemispheres with a blade (e), and transfer them on a small spoon into cold oxygenated ACSF (f). After ~ 5 min equilibration, place one hemisphere on dry tissue with the medial surface up (g). With the help of two spoons remove the diencephalon (h-j). The hippocampus is now visible, as illustrated by the dashed lines (k). Dissect the hippocampus with a spoon out, starting from the fimbria, visible as white structure (l-m). Transfer the hippocampus back into the cold ACSF. Prepare a small agarose-block, position the two hippocampi with the alveus side up and the ventral hippocampus facing the edge of the agar block, and soak carefully all liquid away to allow a good attachment to the agar (n). Glue the hippocampus attached to the agar block onto the ventral part (o).

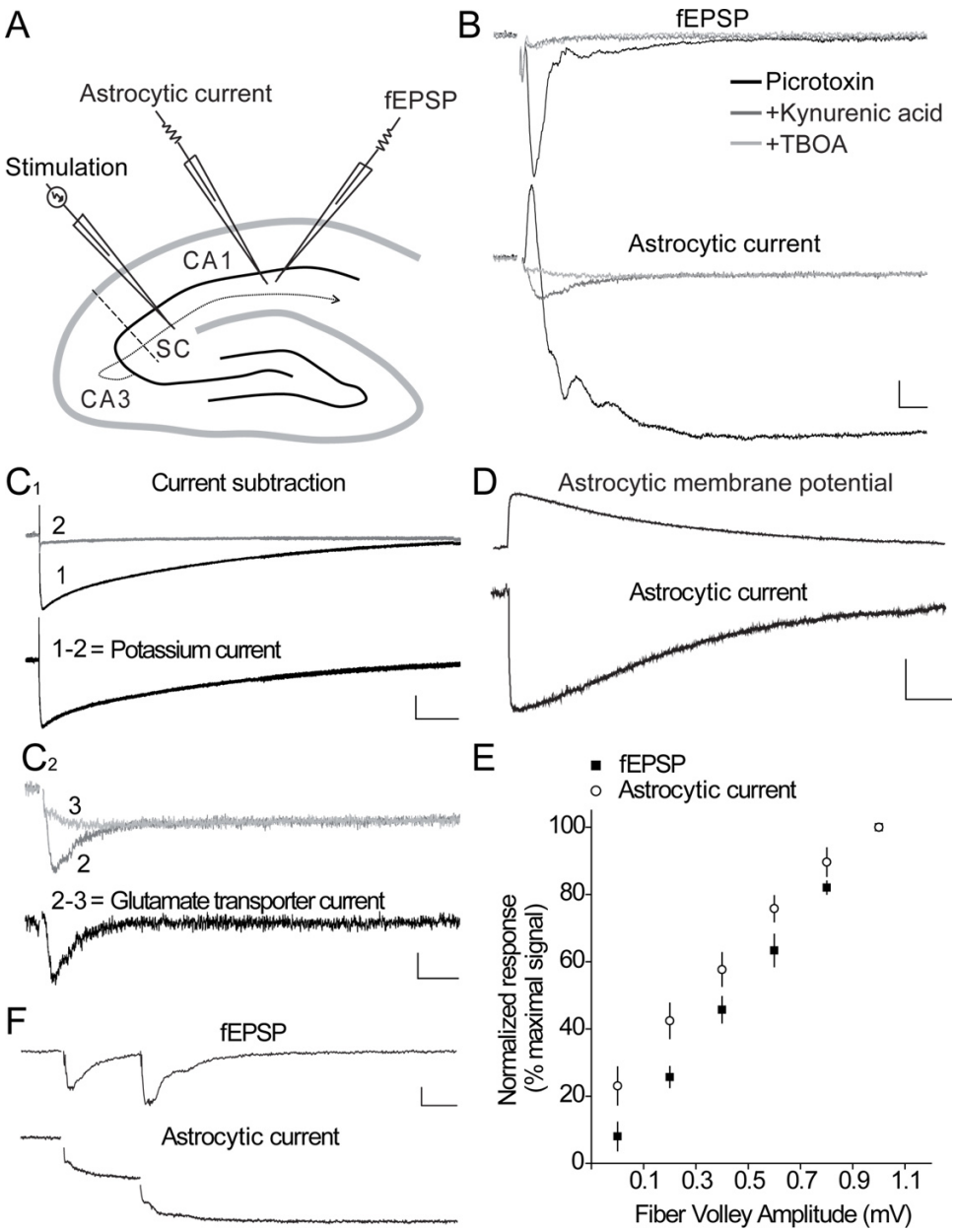

Figure 2. Simultaneous neuronal and astroglial responses evoked-synaptically in the CA1 area of the hippocampus. A) Scheme of the hippocampal slice illustrating the arrangement of the stimulating electrode, to activate the Schaffer collaterals (SC), the patch pipette electrode, to record astrocytic currents, and the extracellular electrode, to record fEPSP, evoked by SC stimulation in the hippocampal CA1 area. B) Representative traces of simultaneous recordings of fEPSPs (upper panel) and astrocytic currents (lower panel) evoked-synaptically by SC stimulation in the presence of pharmacological drugs. The responses are first recorded in the presence of a $GABA_A$ receptor blocker (picrotoxin, 100 μM, black traces) to isolate excitatory responses. Subsequent application of an ionotropic glutamate receptor blocker (kynurenic acid, 5 mM, dark grey traces), inhibits the fEPSP and the major part of the long-lasting astroglial current, unmasking a small and fast transient component of the astrocytic current response, which is sensitive to a glutamate transporter blocker (TBOA, 200 μM, light grey traces). Scale bar, fEPSP 0.1 mV, astrocytic current 15 pA, 10 msec. C_1) Sample trace of the astroglial potassium current (1-2), which can be isolated from the evoked response, shown in B (lower panel, black trace), by subtracting the current component remaining in kynurenic acid (2) from the total current (1). Scale bar, 20 pA, 1 sec. C_2) Sample trace of the glutamate transporter current (2-3), obtained by subtraction of the TBOA insensitive slow component (3) from the current in kynurenic acid (2). Scale bar, 2.5 pA, 25 msec. D) Sample traces of an inward current recorded in voltage-clamp (lower panel) and the corresponding membrane depolarization recorded in current-clamp (upper panel) induced in an astrocyte by SC stimulation. Scale bar, current-clamp 1.5 mV, voltage-clamp 5 pA, 1 sec. E) Input-output curves illustrating the relationship between the presynaptic fiber volleys (input) and the total astroglial current (output) recorded simultaneously in response to SC stimulation (n = 6). The astroglial current increases linearly with the increased fiber volleys, as the neuronal fEPSP. F) Sample traces of the neuronal response (fEPSP) and the astrocytic current are shown for paired-pulse stimulation at a 40 msec interpulse interval. The synaptically-evoked astroglial current exhibits, like neurons, paired-pulse facilitation. Scale bar, 0.1 mV, 5 pA, 20 msec.

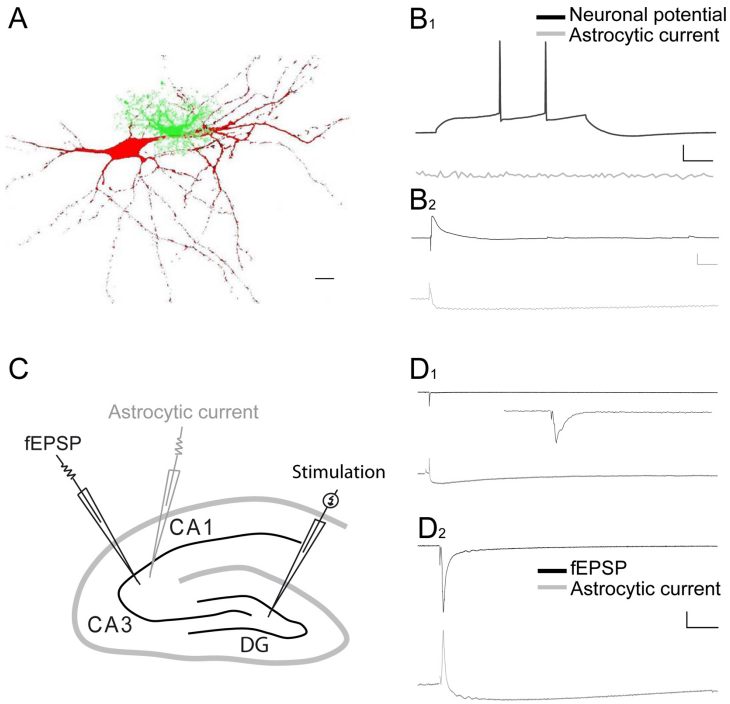

Figure 3. Dual recordings of synaptically-induced neuronal and astroglial responses in the CA1 and CA3 areas of the hippocampus. A) Reconstruction of a CA1 pyramidal cell injected with sulforhodamine-B (red, 0.1 %) and an astrocyte filled with fluorescein dextran (green, 0.1 %), using the whole-cell patch-clamp technique. Scale bar, 10 µm. B_1) Representative traces of simultaneous whole-cell recordings of membrane potentials recorded in current-clamp from a CA1 pyramidal cell and an adjacent astrocyte. Neuronal action potential firing (black trace), evoked by injection of a 20 pA depolarizing current pulse, evokes no response in the adjacent astrocyte (grey trace). Scale bar, 20 mV, 10 pA, 100 msec. B_2) Sample traces of dual whole-cell recordings of a CA1 pyramidal cell in current-clamp and a neighboring astrocyte in voltage-clamp after SC stimulation in the presence of picrotoxin (100 µM). SC stimulation evokes an excitatory postsynaptic potential (EPSP, black trace), associated to a small and long-lasting astrocytic current (grey trace). Scale bar, 5 mV, 10 pA, 100 msec. C) Scheme of the hippocampal slice depicting in the dentate gyrus (DG) and CA3 areas the arrangement of the stimulating electrode, to activate the mossy fibers, the patch pipette electrode (grey), to record astrocytic currents, and the extracellular electrode, to record fEPSP, evoked by CA3 mossy fibers stimulation. D, E) Representative traces of paired recordings of CA3 fEPSPs (upper panels, black traces in D_1 and D_2) and astrocytic whole-cell responses (lower panels, grey traces in D_1 and D_2) to single stimulation of CA3 mossy fibers at 0.02 Hz (zoom in the inset) (D_1) or at 1 Hz stimulation frequency (D_2). Scale bar for D_1 and D_2, 0.2 mV, 15 pA, time: D_1 1 sec; D_2 100 msec. Inset scale bar, 0.1 mV, 100 msec.

Discussion

Dual recording of synaptically-induced neuronal and glial responses is a useful method to study online alterations in pre- and postsynaptic activities associated to changes in astroglial properties. The synaptically-evoked glial membrane depolarization is a direct measure of the extracellular potassium rise[8], due in part to presynaptic action potential firing, but mostly to postsynaptic depolarization [7]. Therefore recordings of glial membrane potential dynamics can be used to investigate modifications in presynaptic excitability, postsynaptic activity, extracellular space volume and potassium buffering capacities[6, 8]. The astroglial glutamate transporter current is a sensitive measure of presynaptic glutamate release, able to monitor short-term changes in release probability[3, 5, 9]. It can furthermore be used to characterize the functional synapse-glia interactions at different synapses or at different developmental stages[10]. It should be highlighted that GLTs are highly temperature sensitive[11] and are driven by the electrochemical gradient of Na^+, K^+ and H^+[12]. Thus the amplitude and kinetics of the GLT current highly depend on the chosen experimental conditions. Furthermore, the actual time course of astroglial glutamate clearance derived from the recorded GLT current is known to be partially obscured. This is due to the filtering of GLT currents by factors such as the electrotonic properties of astrocytes or the asynchronous transmitter release, which distort their kinetics[13]. Methods extracting the temporal features of the filtering mechanisms have been developed and can be used to derive the actual glutamate clearance time course in physiological or pathological situations, as recenly performed[6,13,14]. Additionally, the simultaneous recording of the astroglial membrane depolarization, in current clamp, can provide insights into possible alterations of extracellular potassium transients. Single astrocytes contact up to 100,000 synapses of ~ 100 different neurons, and do therefore integrate and modulate the activity of local neuronal networks.

When using the technique presented here, *i.e.* recording electrophysiological whole-cell responses from astrocytes to gain insights into basal synaptic activity, one should keep in mind that in astrocytes, patch-clamp recordings at the soma level allow detecting currents mostly originating from the cell soma or proximal processes. Indeed, currents detected at the soma only partially originate from fine distal processes when a strong activation of receptors and channels occuring in multiple fine processes can generate currents propagating to the cell soma. Thus basal receptor and channel activity in individual small astroglial processes covering synaptic compartments is hardly detectable. This is due in part to the limited spatial and temporal control of membrane currents and voltages by whole-cell patch-clamp recordings from astrocytes *in situ*. However, it should

be noted that the surface of the abundant tiny astrocytic processes exceeds by far the membrane area of the soma and main processes. In addition, these perisynaptic astroglial microdomains contain the functionally relevant receptors and channels, which likely play an important role in neuroglial communication and synaptic regulation. The technique we presented here is therefore mostly useful to study the astrocytic integration of synchronous activity from neuronal ensembles, occurring in particular during afference stimulation. It should not be used to study the dialogue between individual synapses and adjacent fine astrocytic processes occurring during basal spontaneous activity. An alternative method to study local astroglial responses induced by basal synaptic activity would be to perform patch-clamp recordings from fine processes, as done in dendrites[15]. Although patching these fine astroglial processes is likely challenging due to their small size, it is probably an avenue to pursue to unravel more intimate dialog between astroglial microdomains and individual synapses. However, the likely small electrophysiological astroglial responses resulting from individual fine astroglial processes may be below threshold detection, since electrical noise reaches in average 3-5 pA in patch-clamp recordings. Another method to study astroglial responses to synaptic activity is calcium imaging, since activation of astrocytic membrane receptors or transporters by neuroactive substances can trigger intracellular calcium transients. However, bulk loading of astrocytes with calcium indicators may also mainly reflect somatic activity[16]. The combination of electrophysiology and calcium imaging also enables detecting small calcium signals from fine astroglial processes, either occurring spontaneously or triggered by minimal synaptic stimulation[17, 18]. However, one should keep in mind that high-affinity calcium indicators might act like calcium buffers, inhibiting important calcium signaling pathways, whereas low-affinity indicators might work below detection level. Finally, an elegant and non-invasive technique to study calcium events in fine astrocytic processes, which also circumvents the washout of intracellular signaling molecules during whole-cell patch-clamp, consists in using a membrane targeted calcium sensor, which can be expressed in astrocytes *in situ*, as well as *in vivo*[19]. However, calcium imaging can only provide information about one signaling molecule, which is involved in many, but not all cellular activities, whereas whole-cell patch-clamp provides quantitative information about all the different ionic currents triggered upon channel and receptor activation. Therefore simultaneous electrophysiological recordings from neurons and astrocytes are a unique and powerful method to unravel online the dynamics of neuroglial ionic signalling and its role brain information processing.

Disclosures

No conflicts of interest declared.

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